

U.S. PATENT & TRADEMARK OFFICE  
MAR 30 2006

IFW

1636

TRANSMITTAL LETTER  
(General - Patent Pending)

Docket No.  
16365Z

In Re Application Of: Tom A. Grigliatti, et al.

Application No. 09/896,888	Filing Date June 29, 2001	Examiner Nancy S. Vogel	Customer No. 23389	Group Art Unit 1636	Confirmation No. 3346
-------------------------------	------------------------------	----------------------------	-----------------------	------------------------	--------------------------

Title: INSECT EXPRESSION VECTORS

COMMISSIONER FOR PATENTS:

Transmitted herewith is:

**Claim of Priority Cover Letter**  
**Certified Copy of Canadian Patent Application No. CA 2221819**  
**Certificate of Mailing**

in the above identified application.

- No additional fee is required.  
 A check in the amount of \_\_\_\_\_ is attached.  
 The Director is hereby authorized to charge and credit Deposit Account No. 19-3886/RCT as described below.  
     Charge the amount of \_\_\_\_\_  
     Credit any overpayment.  
     Charge any additional fee required.  
 Payment by credit card. Form PTO-2038 is attached.

**WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.**

  
\_\_\_\_\_  
Signature

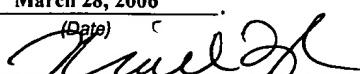
Dated: March 28, 2006

Xiaochun Zhu  
Registration No. 56,311  
Scully, Scott, Murphy & Presser, P.C.  
400 Garden City Plaza-STE 300  
Garden City, New York 11530  
(516) 742-4343

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to the "Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450" [37 CFR 1.8(a)] on

March 28, 2006

(Date)



Signature of Person Mailing Correspondence

Xiaochun Zhu

Typed or Printed Name of Person Mailing Correspondence

cc: XZ:ab

BEST AVAILABLE COPY



**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

**Applicants:** Tom A. Grigliatti, et al.

**Examiner:** Nancy S. Vogel

**Serial No.:** 09/896,888

**Art Unit:** 1636

**Filed:** June 29, 2001

**Docket:** 16365Z

**For:** INSECT EXPRESSION VECTORS

**Dated:** March 28, 2006

**Confirmation No.:** 3346

Commissioner for Patents  
P. O. Box 1450  
Alexandria, VA 22313-1450

**CLAIM OF PRIORITY**

**Sir:**

Applicants in the above-identified application hereby claim the right of priority to Canadian Patent Application No. CA 2221819, filed on January 28, 1998 under 35 U.S.C. §119. Applicants submit herewith a certified copy of CA 2221819.

Respectfully submitted,

Xiaochun Zhu  
Registration No. 56,311

Scully, Scott, Murphy & Presser, P.C.  
400 Garden City Plaza-STE 300  
Garden City, New York 11530  
(516) 742-4343  
XZ:ab

**CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)**

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner For Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on March 28, 2006.

Dated: March 28, 2006

Xiaochun Zhu



Office de la propriété  
intellectuelle  
du Canada

Un organisme  
d'Industrie Canada

Canadian  
Intellectual Property  
Office

An Agency of  
Industry Canada

Bureau canadien  
des brevets  
Certification

La présente atteste que les documents  
ci-joints, dont la liste figure ci-dessous,  
sont des copies authentiques des docu-  
ments déposés au Bureau des brevets.

Canadian Patent  
Office  
Certification

This is to certify that the documents  
attached hereto and identified below are  
true copies of the documents on file in  
the Patent Office.

Specification and Drawings, as originally filed, with Application for Patent Serial No:  
**CA 2221819** on January 28, 1998 by **THE UNIVERSITY OF BRITISH COLUMBIA**,  
assignee of Thomas A. Gigliatti, Dave A. Theilmann, Thomas A. Pfeifer and Dwayne D.  
Hegedus, for "Insect Expression Vectors".

Sylvie Gregoire  
Agent certificateur/Certifying Officer

March 10, 2006

Date

Canada

(CIPO 68)  
31-03-04

O P I C C I P O

## ABSTRACT

The invention provides insect shuttle vectors, and methods of using such vectors, for stably transforming disparate insect cell lines to express heterologous proteins. The invention provides a transformed insect cell selection system based on resistance to the bleomycin/phleomycin family of antibiotics, including the antibiotic Zeocin. Efficient promoters derived from baculovirus immediate early promoters are disclosed for use in directing expression of heterologous proteins, including selectable markers, in transformed insect cells of the invention. Transposon-based vectors are disclosed that provide inducible transposition to optimize heterologous protein expression and unobtrusive markers to facilitate selection of desired transformants.

**What is claimed is:**

1. A shuttle vector for transforming insect cells, comprising:
  - a. a prokaryotic origin of replication;
  - b. an insect promoter having homology to, and capable of functioning as, an immediate early baculovirus promoter;
  - c. a prokaryotic promoter sequence;
  - d. a selectable marker gene capable of conferring resistance to a bleomycin/phleomycin-type antibiotic under the transcriptional control of the insect promoter and the prokaryotic promoter sequence, in insect and prokaryotic cells respectively.
2. The shuttle vector of claim 1, wherein the prokaryotic promoter sequence is a cryptic promoter within the insect promoter.
- 15 3. The shuttle vector of claim 1, wherein the bleomycin/phleomycin-type antibiotic is Zeocin.
4. The shuttle vector of claim 1, further comprising an insertion site for heterologous DNA.
- 20 5. The shuttle vector of claim 4, wherein the insertion site for heterologous DNA is under the transcriptional control of a second insect promoter.

6. The shuttle vector of claim 5, further comprising a heterologous DNA sequence inserted at the insertion site and under the transcriptional control of the second insect promoter.
7. The shuttle vector of claim 1, wherein the insect promoter comprises an IE2B element substantially homologous to SEQ ID NO: 10.
8. The shuttle vector of claim 7, wherein the insect promoter comprises a GATA-IE2B element pair substantially homologous to SEQ ID NO: 9 and SEQ ID NO: 10.
- 10 9. The shuttle vector of claim 8, wherein the insect promoter comprises a sequence substantially homologous to SEQ ID NO: 1 from bp 351 to bp 527.
10. The shuttle vector of claim 9, wherein the insect promoter comprises a sequence substantially homologous to SEQ ID NO: 1.
- 15 11. The shuttle vector of claim 1 further comprising DNA transposable elements defining a transposon.
12. The shuttle vector of claim 11, wherein the selectable marker gene is within the transposon.

13. The shuttle vector of claim 12, further comprising an insertion site for heterologous DNA within the transposon.
14. The shuttle vector of claim 13, further comprising a heterologous DNA sequence inserted at the insertion site and under the transcriptional control of a second insect promoter.  
5
15. The shuttle vector of claim 11, further comprising an inducible transposase gene within the transposon.  
10
16. Insect cells transformed with the shuttle vector of claim 1.
17. Insect cells transformed with the shuttle vector of claim 11.  
15
18. A method of transforming insect cells comprising:
  - a. inducing the insect cells to take up an insect shuttle vector comprising a selectable marker gene under the transcriptional control of an insect promoter, the selectable marker gene being capable of conferring resistance to a bleomycin/phleomycin-type antibiotic, and the insect promoter having homology to, and being capable of functioning as, an immediate early baculovirus promoter; and,  
20
  - b. selecting transformed cells that are resistant to the bleomycin/phleomycin-type antibiotic.

19. A method of increasing the copy number of a heterologous DNA sequence in a recombinant cell, comprising:
- a. providing a recombinant cell having,
    - i. the heterologous DNA sequence positioned within a transposon defined by DNA transposable elements;
    - ii. an unobtrusive marker gene linked to the heterologous DNA sequence within the transposon;
    - iii. a transposase gene, the transposase being capable of mediating replicative transposition of the transposon;
  - b. permitting expression of the transposase gene to mediate replicative transposition of the heterologous DNA sequence; and,
  - c. monitoring the increase in copy number of the heterologous DNA sequence by monitoring the expression of the unobtrusive marker gene.
- 15 20. The method of claim 19 wherein the transposase gene is inducible and the step of permitting expression of the transposase gene comprises inducing expression of the transposase gene.
21. Recombinant insect cells transformed with the shuttle vector of claim 1, expressing a heterologous protein selected from the group consisting of melanotransferrins and biologically active derivatives thereof.

22. A heterologous protein produced by the cells of claim 21, selected from the group consisting of melanotransferrins and biologically active derivatives thereof.
23. Recombinant insect cells transformed with the shuttle vector of claim 1, expressing a heterologous protein selected from the group consisting of insect ion transport peptide hormones and biologically active derivatives thereof.  
5
24. A heterologous protein produced by the cells of claim 23, selected from the group consisting of insect ion transport peptide hormones and biologically active derivatives thereof.  
10
25. Insects comprising cells stably transformed with the vector of claim 1.
26. A recombinant cell comprising a heterologous, inducible transposase gene, wherein transposase is expressed in the cell at levels that mediate transposition only upon induction of the transposase gene.  
15

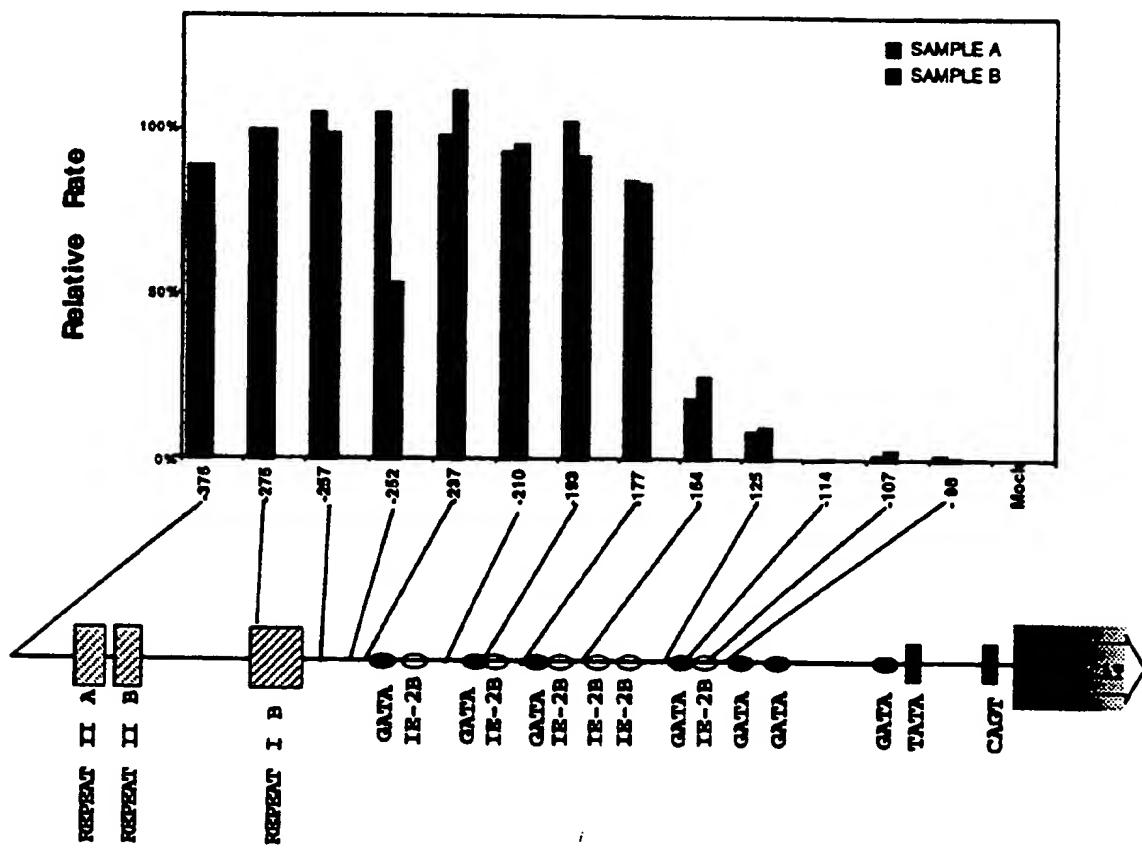


Figure 1

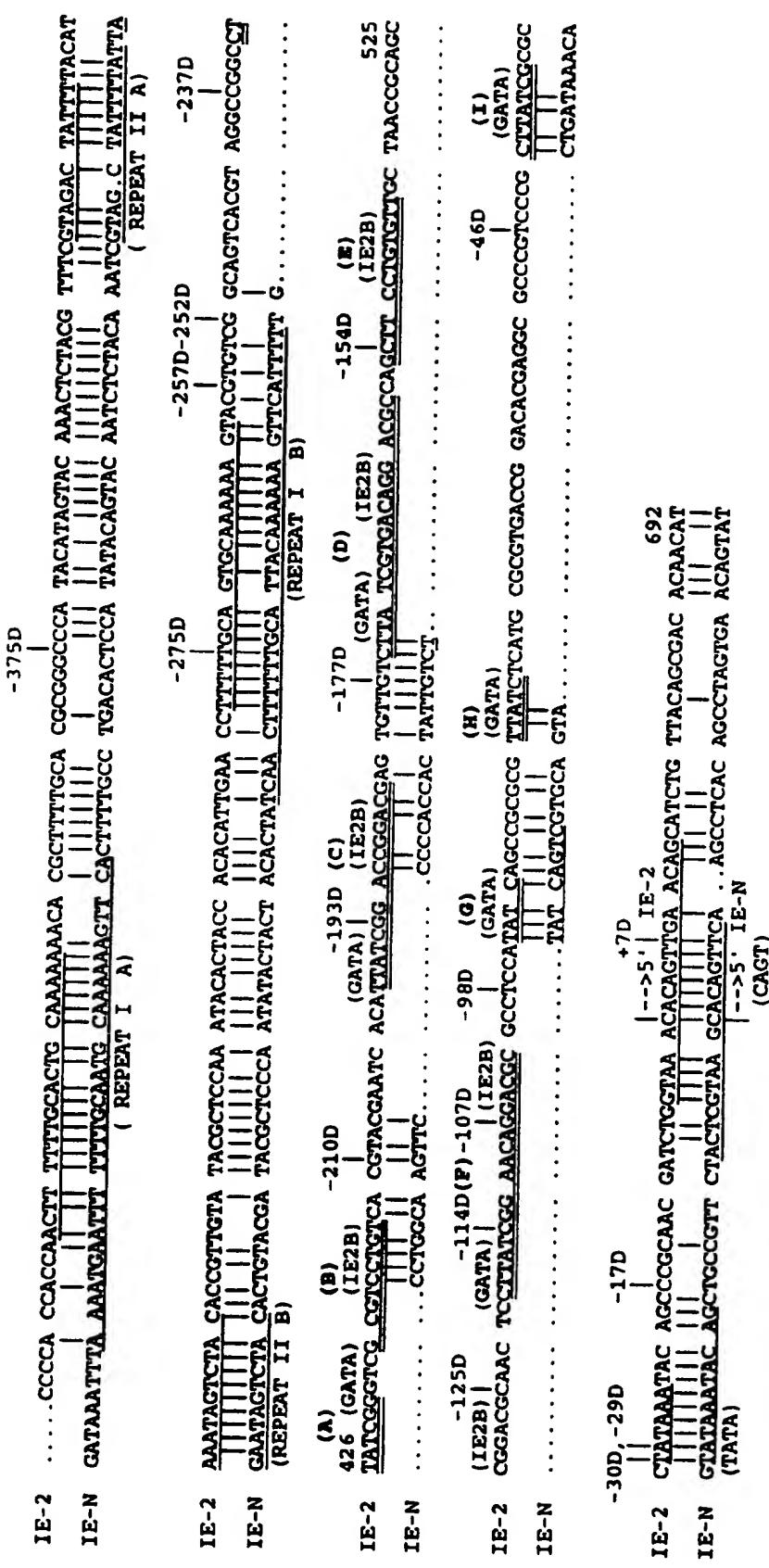


Figure 2a

A	-232	C T T A T C G G	G A C A G G A C G C	-225
B	-212R	T T A T C G G	A C <span style="border: 1px solid black; padding: 0 2px;">C</span> G G A C G C	-221R
C	-197	C T T A T C G <span style="border: 1px solid black; padding: 0 2px;">T</span> G	A C A G G A C G C	-191
D	-175	C T T A T C G G A	G A C G G A C G C	-162
E	-147R	C T T A T C G G A	G A C A G G A <span style="border: 1px solid black; padding: 0 2px;">A</span> G C C	-157R
F	-118	C T T A T C G G A	A A C A G G A C G C	-101
G	-93	C T T A T C G G A	A C A G G A C G C	-89
H	-80	T T A T C G		-76
I	-40	C T T A T C G		-34
Consensus		C T T A T C G G -	A C A G G A C G C	

Figure 2b

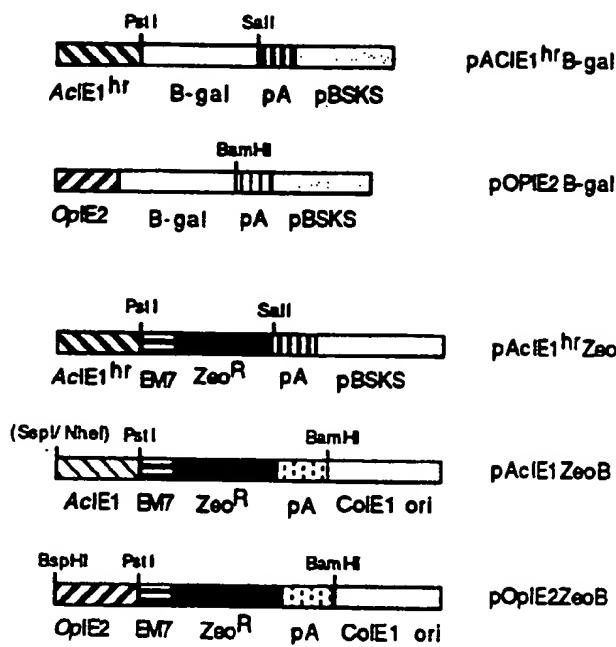


Figure 3

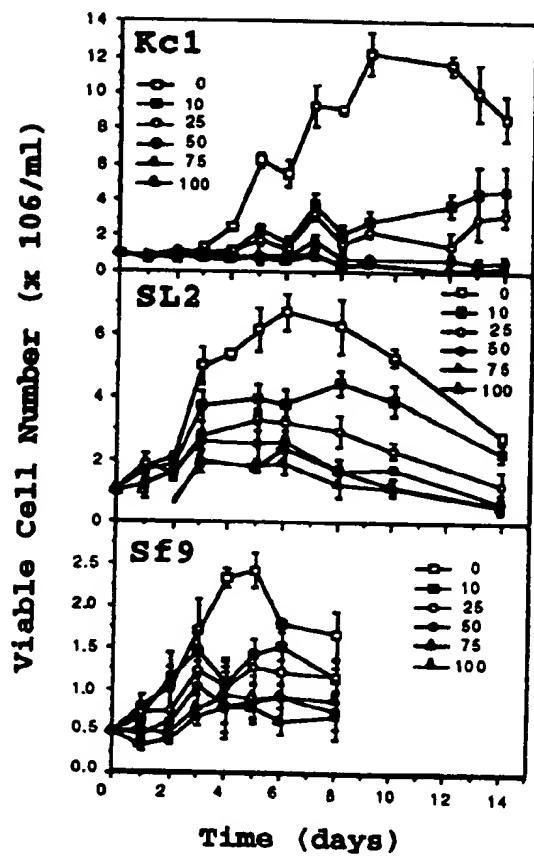
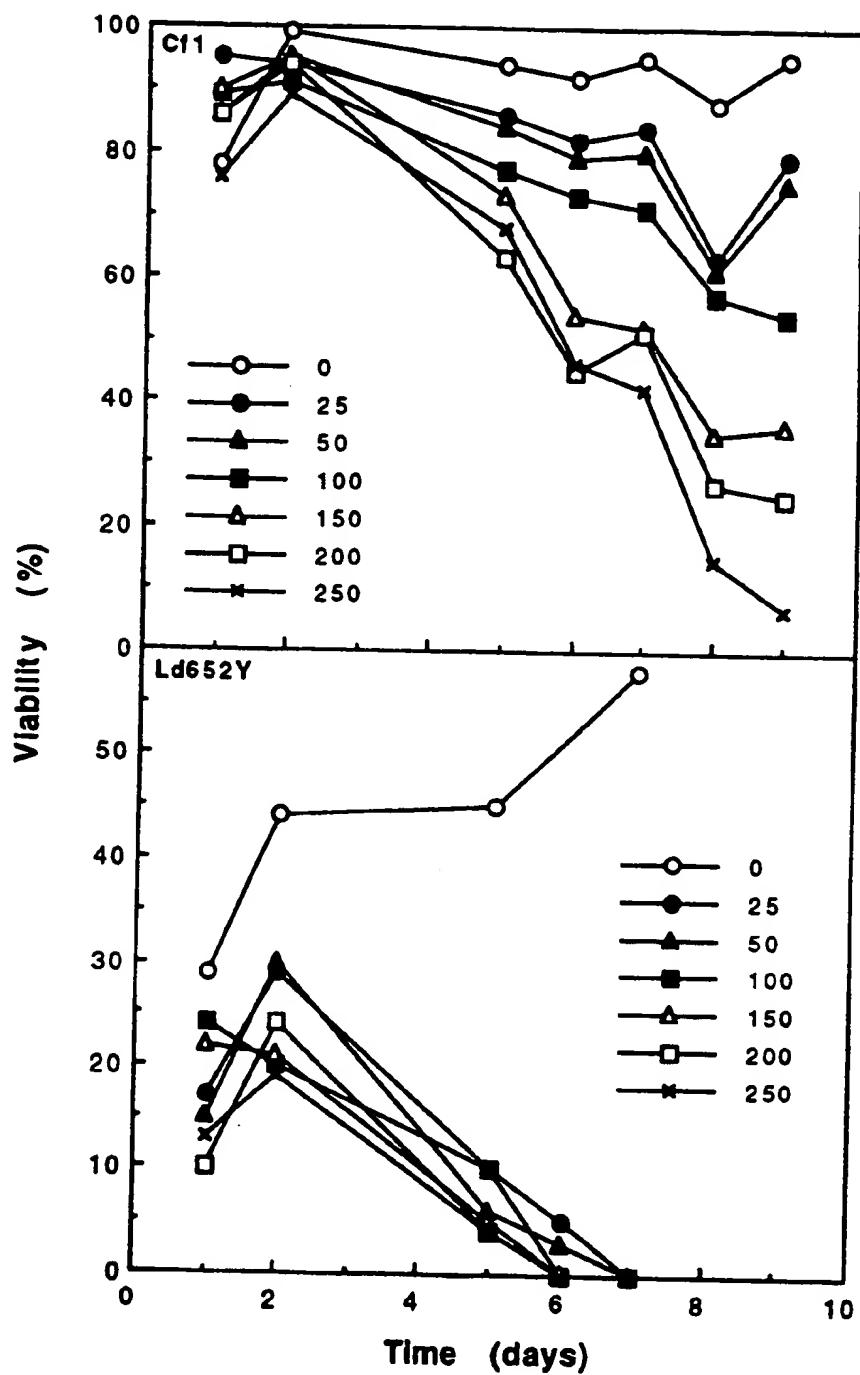
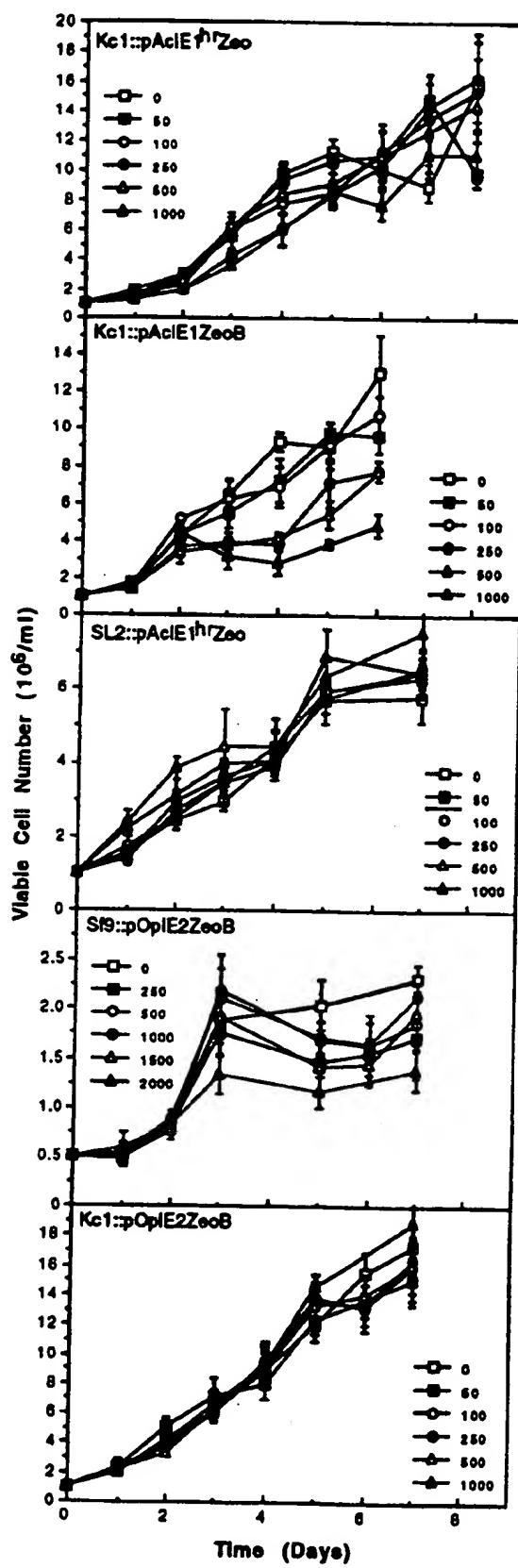


Figure 4a

**Figure 4b**

**Figure 5**

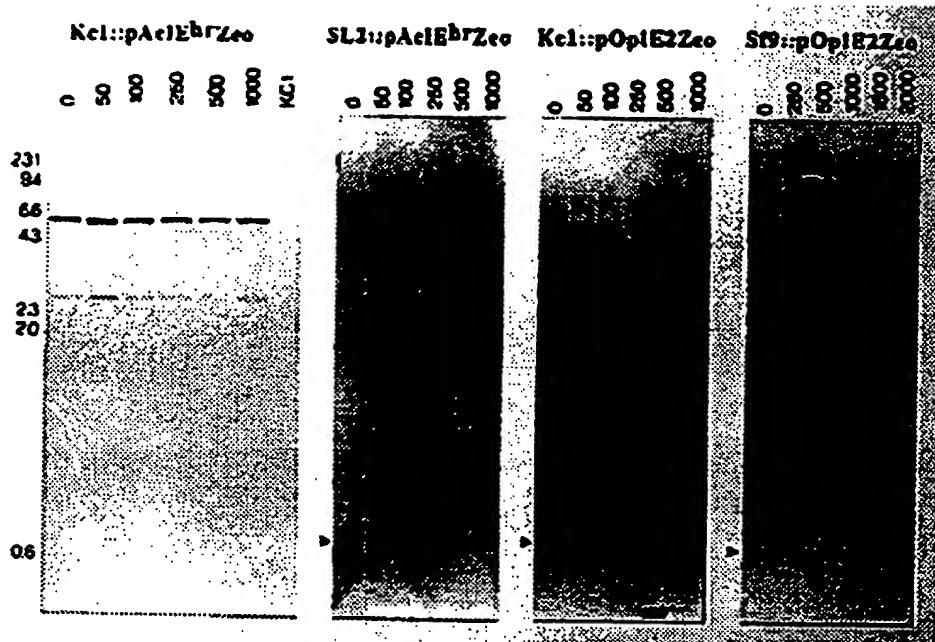


Figure 6

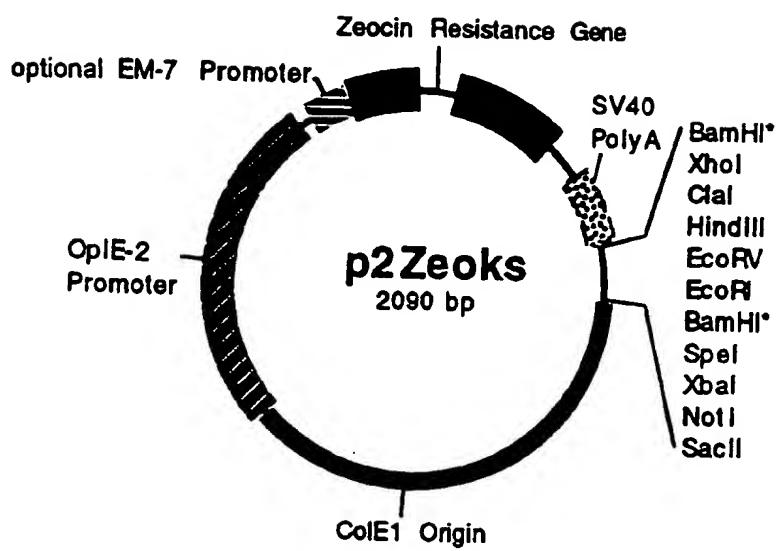
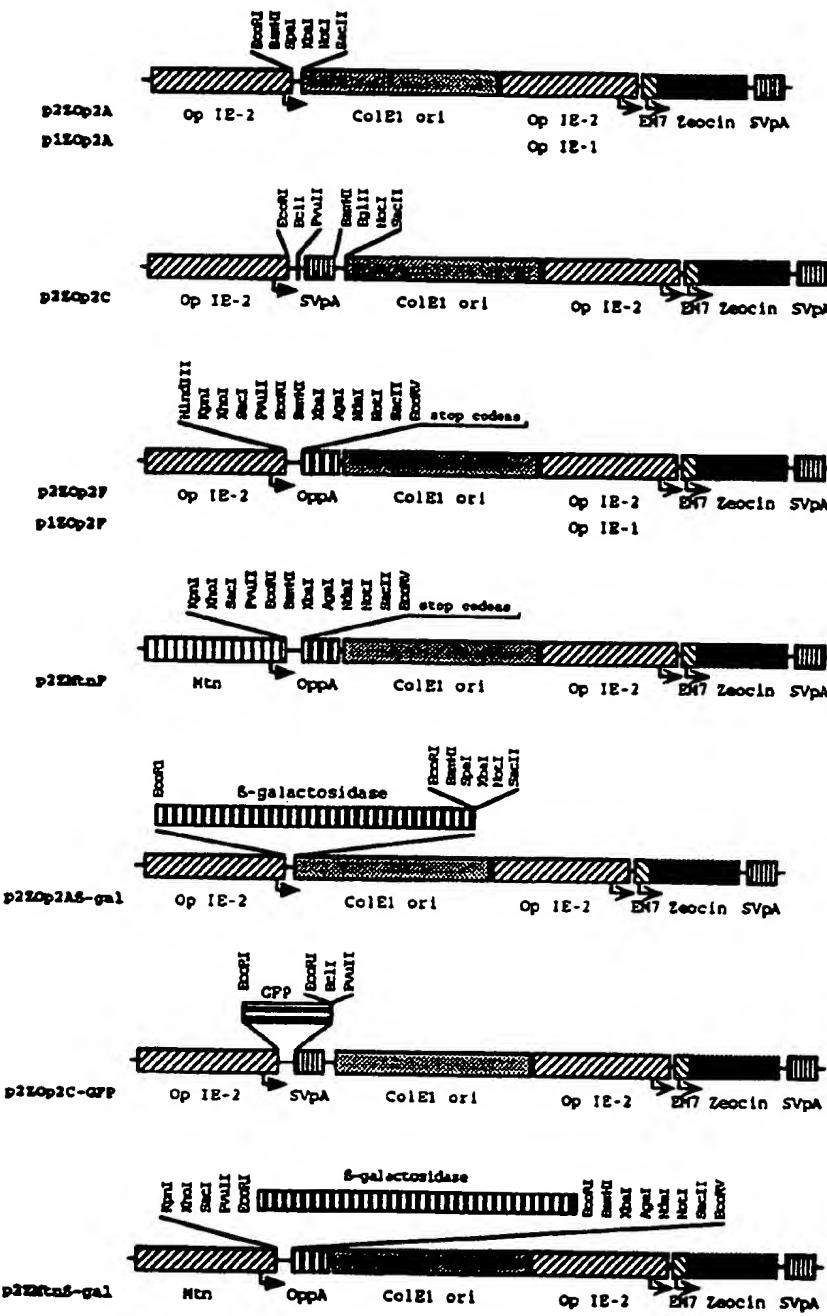


Figure 7



**Figure 8**

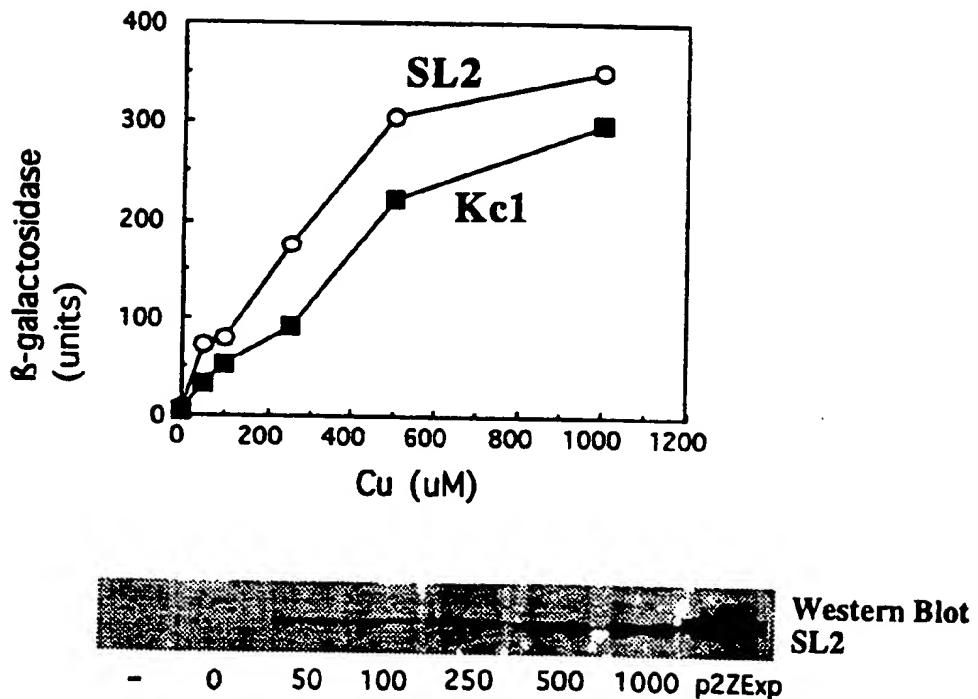
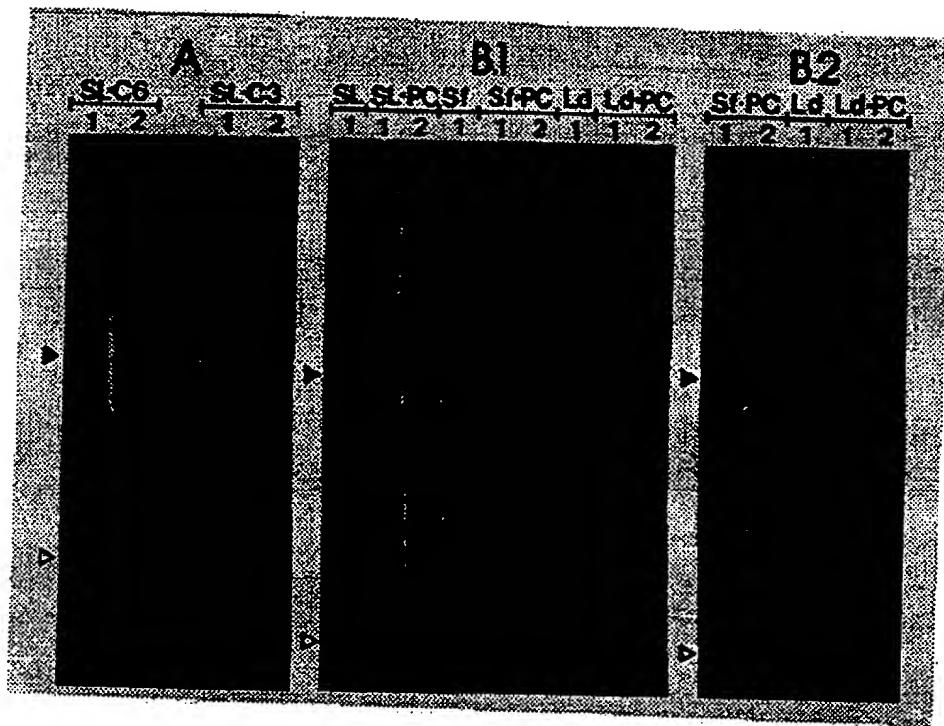


Figure 9



**Figure 10**

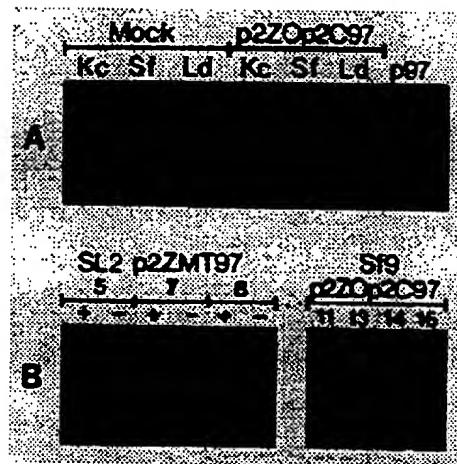
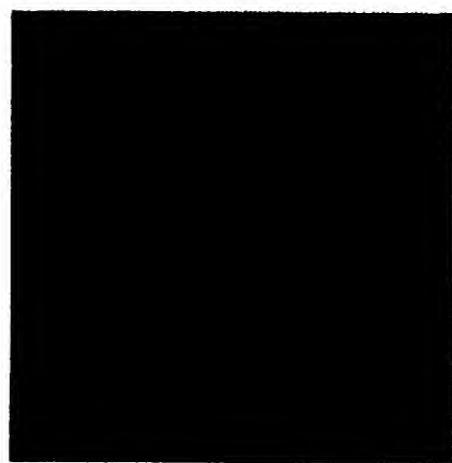


Figure 11

CA 02221819 1998-01-28



**Figure 12**

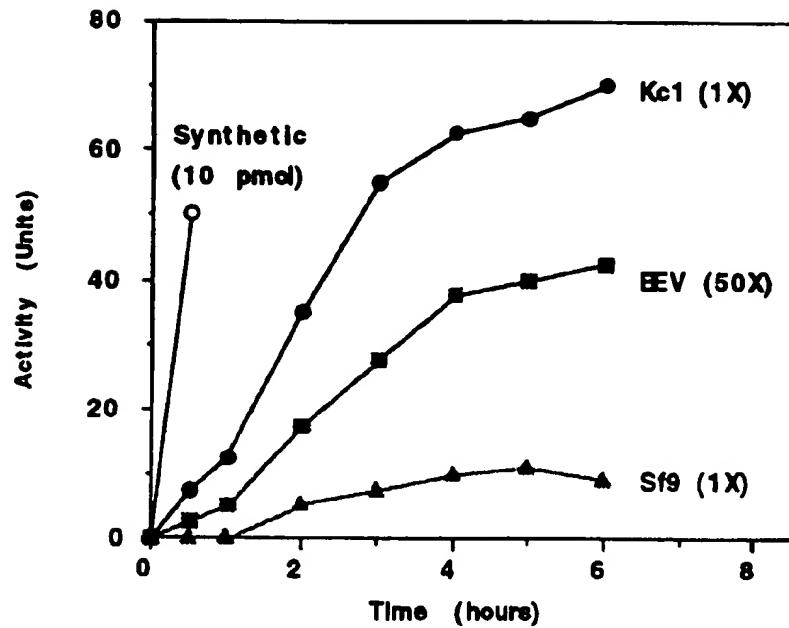
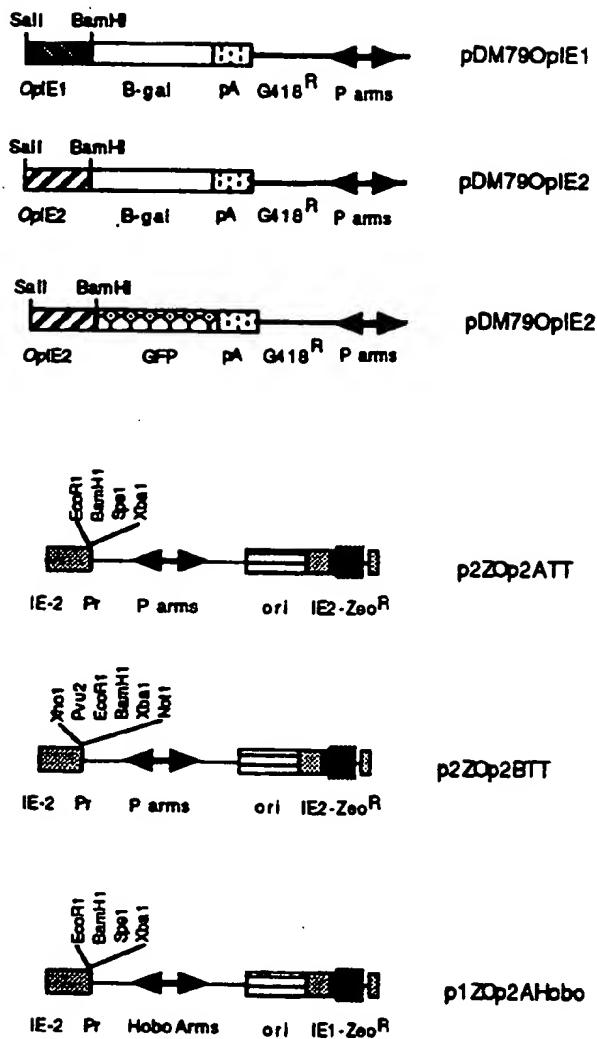


Figure 13

**Figure 14**

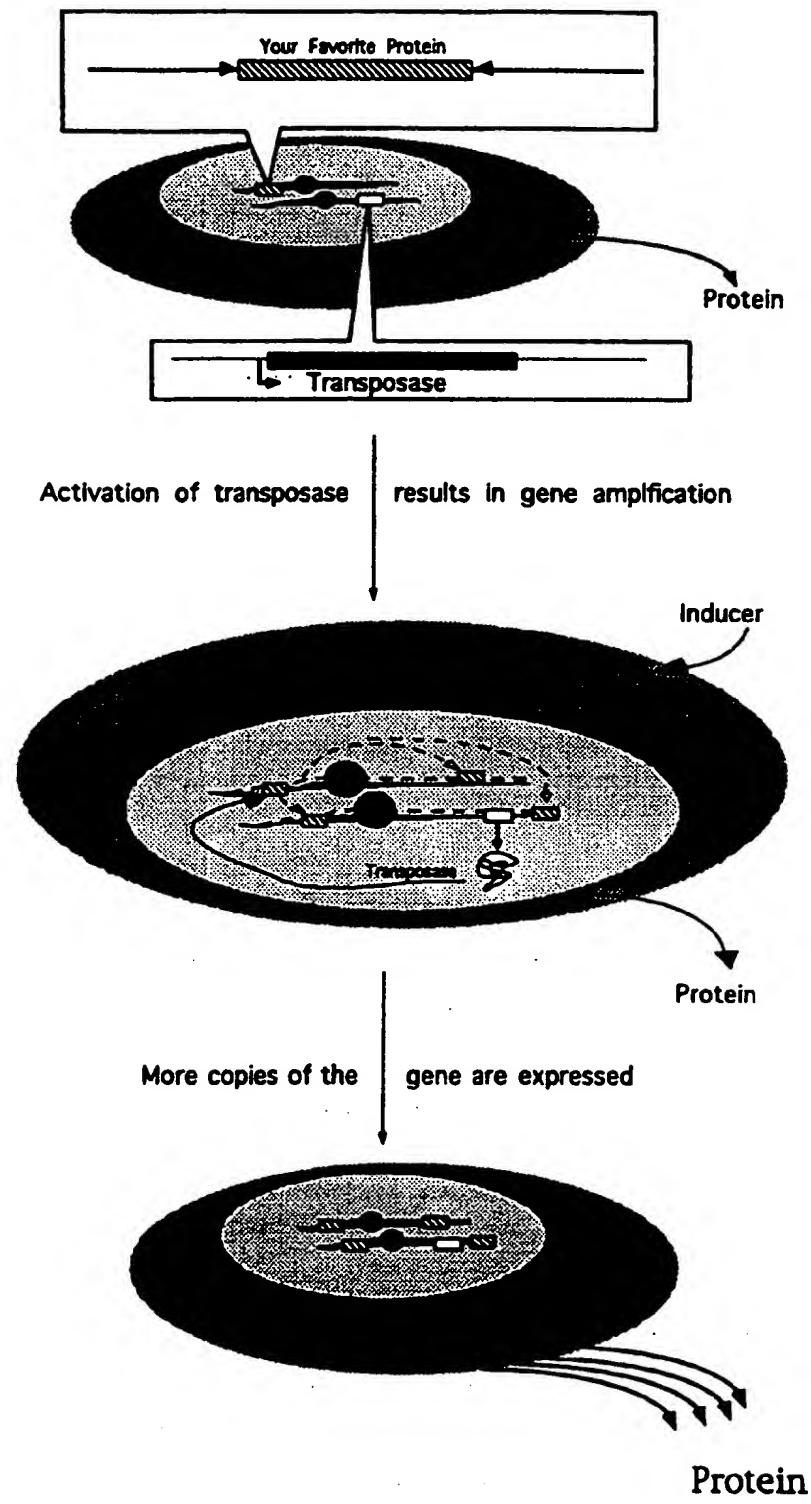


Figure 15

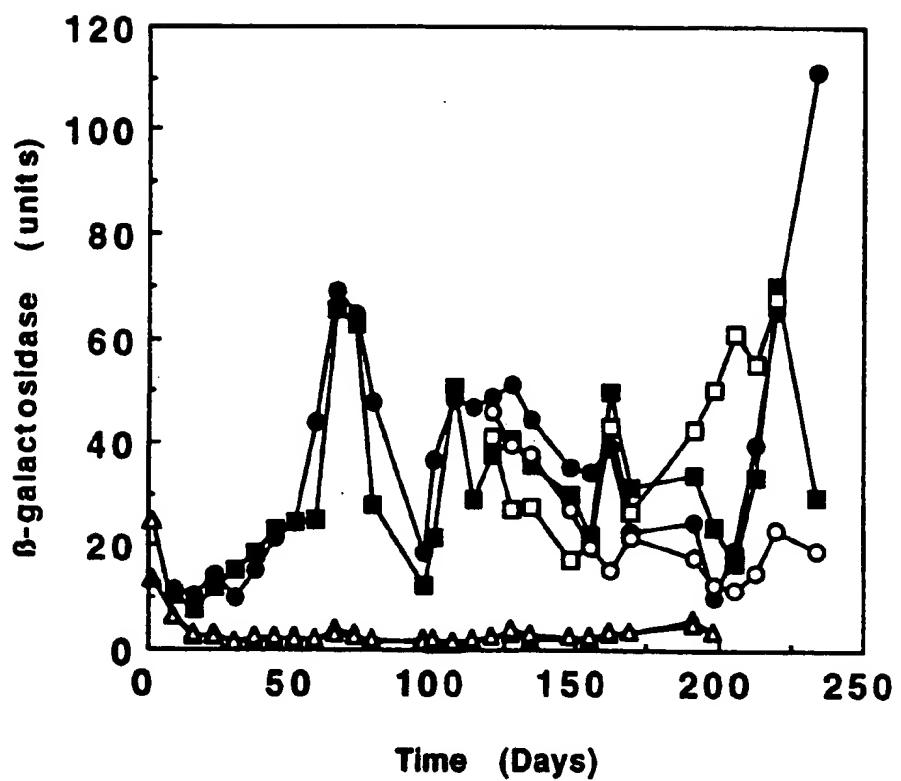


Figure 16

CA 02221819 1998-01-28

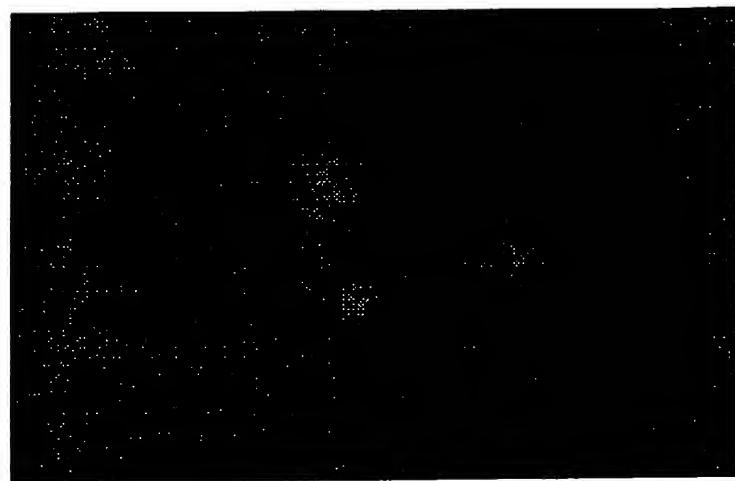


Figure 17

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- BLACK BORDERS**
- IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- FADED TEXT OR DRAWING**
- BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- SKEWED/SLANTED IMAGES**
- COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- GRAY SCALE DOCUMENTS**
- LINES OR MARKS ON ORIGINAL DOCUMENT**
- REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- OTHER:** \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**